A WATER-SOLUBLE FORM OF ERGOSTEROL AND CHOLESTEROL FOR PHYSIOLOGICAL STUDIES

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The investigation of the role of ergosterol in the physiology of Saccharomyces cerevisiae has been hampered by the necessity of adding the water insoluble sterol to growth media and cell free reaction mixtures by suspension in a variety of surface active agents (Festenstein, 1955; Parks and Starr, 1963; Katsuki and Bloch, 1967). The occurrence of a soluble, physiologically active form of ergosterol has been investigated in this laboratory. The data communicated here arose from the observation that ergosterol added to certain growth media became refractory to petroleum ether extraction. The analysis of the capacity of the constituents of these media and other solutions to bind ergosterol is the subject of this paper. A simple, highly efficient mechanism for the solubilization of sterol in aqueous media is described.

MATERIALS AND METHODS

Ergosterol and cholesterol used in these studies were purchased from Sigma Chemical Co. and were recrystalized from ethanol prior to use. Sterol additions to solutions were made from ethanolic solutions. Extraction of sterols from samples were performed with a minimum of three volumes of petroleum ether B.P. 30°-60°. The extracts were then evaporated to dryness under nitrogen and the sterol content of the dried

extracts determined using a slightly modified Liebermann-Burchard color test (Starr and Parks, 1962). Tryptone and yeast extract were products of Difco Laboratories. Media used were: YCM consisting of 2% glucose, 2% tryptone and 1% yeast extract, and Wickerham's media (Wickerham, 1946).

RESULTS

1. Binding of Ergosterol by Constituents of YCM Medium. It has been observed that ethanolic solutions of ergosterol added to culture media resulted in an inability to re-extract the sterol with petroleum ether. A series of experiments was designed to test which constituents of the medium effected the binding. The results are shown in Table 1. It may be seen that as much as 94% of the proferred sterol became resistant to solvent extraction from the YCM medium and that almost all of this binding could be accounted for by the presence of yeast extract.

TABLE 1: Binding of Ergosterol by Constituents of Yeast Growth Media.

Flask Contents	1 µg Sterol Recovered	2 µg Sterol Recovered
YCM Medium	12	32
2% Glucose	126	143
2% Tryptone	75	65
1% Yeast Extract	18	35
Distilled H_2O	200	188
Phosphate Buffer	179	182
Phosphate Buffer + 2% Glucose	182	185
Wickerham's Medium	143	144

Each flask contained 100 ml of the indicated solution to which was added 200 ug of ergosterol in 5.0 ml of ethanol. Column one and column two differ in that ergosterol was added prior to and subsequent to autoclaving respectively. Sterol was extracted with successive 40,20,20, and 10 ml volumes of petroleum ether. Phosphate buffer consisted to 6.5318 g K_2HPO_4 and 8.5062 g KH_2PO_4 per liter to give a pH of 6.6.

2. Binding of Ergosterol and Cholesterol by Solutions of 5% Yeast Extract. In order to investigate the binding of sterols by yeast extract, increasing quantities of ergosterol and cholesterol were added to 10 ml volumes of 5% yeast extract, the tubes mixed, autoclaved and extracted with three 10 ml volumes of petroleum ether. The amounts of sterol recovered are shown in Table 2 and demonstrate the capacity of yeast extract to bind substantial quantities of both ergosterol and cholesterol.

TABLE 2: Binding of Ergosterol and Cholesterol by Solutions of 5% Yeast Extract

Sample	µg Sterol Added	µg Recovered*	µg Bound	% Bound
Ergosterol-1	100	8	92	92
Ergosterol-2	250	3	247	99
Ergosterol-3	400	13	387	97
Ergosterol-4	500	16	484	97
Cholesterol-1	100	4	96	96
Cholesterol-2	250	13	237	95
Cholesterol-3	400	17	383	96
Cholesterol-4	500	23	477	96

* Corrected for sterol of a yeast extract blank.

3. Binding of Ergosterol by Solutions of 3% Cas-Amino Acids. In order to determine whether or not the amino acids present in growth media were responsible for the binding of sterol, the binding capacity of a 3% solution of vitamin free Cas-amino acids (Difco Laboratories) was determined, using the procedure described under Table 1. The results demonstrate nearly quantitative recovery of the sterol. The Cas-amino acids bind less than 6% of the supplied sterol.

4. Recovery of Sterol from Yeast Extract by Extraction and Hydrolysis. It was of interest to determine whether or not the added sterol could be

recovered by extraction of the unbound sterol and release of the bound sterol to free sterol by means of acid and base hydrolysis. The results of such an experiment are given in Table 3 and demonstrate that in excess of 91 percent of the added sterol can be recovered by extraction and methanolic pyrogallol saponification.

TABLE 3: Recovery of Sterol from Yeast Extract by Extraction and Hydrolysis

Sample	Hydrolysis Method	µg From Wash	µg From Hydrolysis	Total µg Recovered
1	Acid	50.5	95.5	146
2	Base	50.5	40.5	91
3	Pyrogallol	50.5	408.0	458.5

Six tubes containing 10 ml volumes of 5% yeast extract to which had been added 500 µg quantities of ergosterol were extracted with three 10 ml volumes of petroleum ether and the sterol content of the extracts determined. The tube contents were then hydrolysed by the three methods described below and the extractable sterol content determined. The average value is reported here.

1) 10 ml of 0.2N HCL were added, the tubes heated in an Arnold sterilizer for 1 hr, the pH adjusted to 10, and the tubes extracted.

2) 10 ml of 40% KOH were added, the tubes heated for 6 hrs in an Arnold sterilizer and extracted.

3) 3.0 ml of 0.5% pyrogallol in absolute methanol, 2.0 ml of 60% KOH and 3.0 ml of absolute methanol were added and the tubes refluxed with cold fingers for 1 hr and extracted.

DISCUSSION

The data presented here demonstrate that yeast extract is capable of binding exogenous sterol, making it refractory to extraction with petroleum ether and that this bound sterol can be recovered by methanolic pyrogallol saponification. The solubility of ergosterol in yeast extract and the ease of formation of the complex greatly facilitates experiments in which sterols are to be provided to aqueous environments. Since yeast extract is a constituent of many culture media, its inclusion allows the solubilization of sterols yet obviates the requirement for various surface active agents. Using this soluble ergosterol complex, studies are in progress to assess the role of ergosterol in the metabolism of yeast.

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